REMARKS

Claims 1, 11-16, 21, 22, 24-31, 37-39 and 42 are pending in the application. Claims 2-10, 23, 32-36, 40 and 41 have been canceled without prejudice.

In the Office Action, Applicants can find no recitation of a rejection of Claim 23 or any reason that the Office Action has rejected Claim 23. As such, Applicants are proceeding in their response with the assumption that Claim 23 has not been rejected.

The Specification has been amended at Page 5. The 3 paragraphs from line 6 to line 25 incorrectly identify the figures as 1A, 1B, and 1C respectively. This amendment corrects this typographical error and renumbers the figures as 2A, 2B, and 2C respectively. By this amendment no new matter has been added.

The Specification has been further amended at page 41, to correct the typographical error by the term "core phenyl-thiozol-benzamide structure" with the term "core *N*-(4-phenylthiazol-2-yl)benzamide structure." This paragraph has been further amended to correct the typographical error wherein the recited compounds are found in Figure 16 instead of Figure 17. By these amendments no new matter has been added.

The preamble of Claim 1 has been amended to remove the phrase "or preventing." Claim 1 has also been amended to recite that the method involves administering "to a subject a compound comprising a core *N*-(4-phenylthiazol-2-yl)benzamide structure." Support for this amendment can be found in Figure 16, wherein the exemplified compounds that are described at page 41, lines 8 to 17, i.e., IBT 13131 and IBT 14664. Claim 1 has further been amended to delete the phrase "inhibiting the interaction between Hec1 protein and at least one further protein by."

Claim 21 has been amended to recite that the "at least one further protein" is "Hint1 protein" thereby incorporating into Claim 21 the limitation recited in Claim 23 which has not been rejected in the present Office Action. As such, the term "at least one further protein" has been replaced by "Hint1 protein" in all occurrences. Claim 21 has been further amended to recite that Hec1 is contacted "with" Hint1 protein.

Claim 22 has been amended to recite that the method of Claim 21 further comprises contacting Hec1 with Nek2 protein. Support for this amendment is found in original Claim 21.

Claim 23 has been canceled.

Claim 24 has been amended to recite "the Hint1 protein" instead of "at least one further protein."

Claim 25 has been amended to recite "the Hint1 protein" instead of "at least one of the further proteins."

Claim 27 has been amended to recite "the Hindt1 protein" instead of "the further protein."

Claim 28 has been amended to recite in step (b) that the amount which the molecules or combination of molecules "interferes with a function of Hec1 protein, Nek2 protein and/or Hint1protein involved" in cell proliferation, etc. is that which is being identified. This amendment incorporates into step (b) the purpose recited in the claim's preamble.

Claim 37 has been amended to depend from Claim 1 and therefore has been rewritten as a method claim. Claim 37 has also been amended to recite that the compound having a core *N*-(4-phenylthiazol-2-yl)benzamide structure is *N*-[4-(2,4-dimethylphenyl)thiazol-2-yl)benzamine or *N*-[4-(2,4,6-trimethylphenyl)thiazol-2-yl)-2,4-dimethoxybenzamine.

The preamble of Claim 38 has also been amended to recite the term "compound" instead of "moleculeor ligand." Claim 38 has also been amended to recite that compound comprising the composition is a compound identified by the method of Claim 21. This amendment is necessitated by the cancellation of Claim 36 from which Claim 38 originally depended.

Claim 39 has been amended to recite that the compound of Claim 1 "can have additional groups on the benzene rings." Support for this amendment is found at page 42, lines 14-15.

By these amendments, no new matter has been added.

REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

The Office Action has rejected Claims 1-5, 8, 11-19 and 39-42 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants believe the amendments herein to Claims 1 and 39-41 overcome this rejection.

Specifically, Claim 1 has been amended to delete the term "small molecule drug" and replace this term with the phrase "a compound comprising a core *N*-(4-phenylthiazol-2-yl)benzamide structure" that describes the compounds recited in the specification at page 41, lines 8 to 17 and that are exemplified in Figure 16.

Similarly, Claim 39 has been amended to delete the term "small molecule drug" and replace this term with the phrase "the compound has additional groups on the benzene rings." Support for this amendment can be found in the Specification at page 41, lines 14-15.

Claims 40 and 41 been canceled thereby rendering the rejection to these Claims moot.

By these amendments to Claims 1 and 39-41, Applicants believe the Office Action's rejection of Claims 1-5, 8, 11-19 and 39-42 under 35 U.S.C. § 112, second paragraph, have been overcome. Therefore reconsideration and withdrawal of the rejection of the claims under35 U.S.C. § 112, second paragraph, is respectfully requested.

REJECTION UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

The Office Action has rejected Claims 1-5, 8, 11-20 and 36-42 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement, in that the claim(s) contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants respectfully disagree.

The Office Action states that "Claims 1-5, 9, 11-20 and 39-42 are drawn in part to a method for treating a disease involving cell hyperproliferation, including cancer and stenosis, comprising the administration of a small molecule drug which inhibits the interaction between Hec1 protein and at least one further protein." The Office Action asserts that the "specification teaches that out of eight molecules, only two molecules showed the ability to kill dividing HeLa cells (page 41, lines 8-18)...Thus, the disclosure of inhibiting the interaction between Hec1 and Nek2 is not commensurate in scope with the inhibition of Hec 1 and at least one other protein. Further, the toxicity of IBT13131 and IBT14664 on HeLa cells does not translate into a method of treating patients for cancer or for stenosis."

Claims 17-20 relating to a method for treating stenosis have been cancel thereby obviating this reason for rejecting those Claims. In addition, Claim 1 has been further amended to delete the phrase that the method involves inhibiting the interaction between Hec1 and at least one further protein. As such, Claim 1 now recites that the method relates to treatment of a disease in a subject involving hyperproliferation. This is accomplished by administering a compound comprising a core *N*-(4-phenylthiazol-2-yl)benzamide structure.

Applicants have shown by the HeLa cell assay that the disclosed compounds IBT13131 and IBT14664 are capable of successfully stopping cell proliferation in cancer cells. The Office Action's statement that "the specification teaches that out of eight molecules, only two molecules showed the ability to kill dividing HeLa cells" is a misunderstanding of the disclosure. What Applicants have disclosed is that out of 40,000 chemicals tested in a preliminary screening, eight compounds were shown to "promote yeast growth at concentration of about 10 μM" (page 40, lines 30-31). As such, these eight compounds successfully passed a preliminary screening that indicates these eight compounds, by disrupting the interaction of Hec1 and Nek2 in the reverse yeast two-hybrid screening, are potential candidates for testing in cancer cells, i.e., HeLa cells.

The eight "candidate" compounds were then screened in the HeLa cell assay and two compounds, IBT13131 and IBT14664, were found to have "significant activity in killing dividing cells" (page 41, line 11). Moreover, Applicants have not just identified two active compounds, but have also identified a core *N*-(4-phenylthiazol-2-yl)benzamide structure (page 41, lines 14-16) necessary for activity. This is because the "remaining six compounds that showed no activity [in the HeLa assay] did not share the same core structure." As such, Applicants have shown the following:

1. A yeast-based test for preliminary screening of compounds based upon mammalian cell Hec1 (See, the disclosure beginning at page 36, line 5);
2. This test can be used to vet potential drug candidates from a large library of molecules (in the instant case, 8 molecules from 40,000 screened. See, page 40, lines 28-31); and
3. Candidates from the initial screening can then be subjected to a more specific HeLa mammalian cell screening to test whether the candidate compounds inhibit cell proliferation of cancer cells *in vitro*, and are therefore potential drug candidates.

Applicants have thus narrowed down the required structural features necessary for activity thereby providing the artisan with a rational place to begin the search for other potential drug candidates useful for treating a disease involving cell proliferation.

The Office Action also summaries the disclosure of Mohanlal (WO 2002/40717) stating that the "reason for the high failure rate in clinical trials is the poor predictive value of currently

used screening technologies for biological validation, etc." as it relates to "clinical trials involving humans." Applicants agree with Mohanlal in that a potential drug candidate that has been found to be efficacious even in recognized and established *in vivo* animal tests may not be found to be successful drug candidates when tested in human clinical trials. That fact, however, is not germane to the present issue. An applicant for a patent is not required to have conducted human clinical trials in order to obtain valid claims to the disclosed subject matter. Establishing the efficacy of a drug in human clinical trials and/or whether a drug candidate progresses forward for use in humans is not a prerequisite for seeking patent protection of a discovery.

The Office Action further states that the claims are "drawn in part to a method for preventing a disease involving cell hyperproliferation" and that the "specification fails to teach how to identify patients who are about to develop the hyperproliferative disease." Claim 1 has been amended to delete the term "preventing." As such, Applicants believe this amendment overcomes this reason for the Office Action's rejection of the claims.

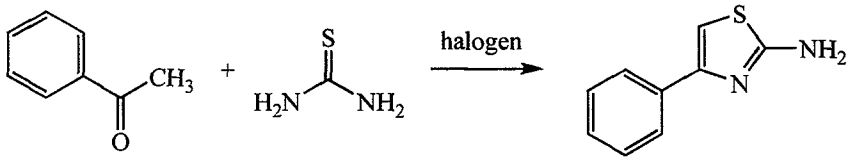
As it relates to Claim 37, the Office Action states that this claim "is drawn in part to the molecules which had no activity in mammalian cells." Claim 37 has been amended to delete compounds IBT4282, IBT6432, IBT11830, IBT12008, and IBT15154. As such, Applicants believe this amendment overcomes this reason for the Office Action's rejection of the claims.

The Office states that Claim 36 is "drawn to a molecule or ligand identified by the method of claim 21 wherein said molecule or ligand lessens proliferation when contacted with proliferating cells." Further to Office Action states "Claim 38 is drawn to a composition comprising the molecule or ligand of claim 36 and a pharmaceutically acceptable carrier...Thus the specification fails to enable the cope of a "molecule or ligand" of claims 36 and 38." As it relates to Claim 36, cancellation of Claim 36 obviates the rejection over this claim. Claim 38 has been amended to recite a "compound identified by the method of Claim 21, that comprises a core *N*-(4-phenylthiazol-2-yl)benzamide structure" and therefore not only encompasses compounds that are identified by the method of Claim 21, but compounds that comprise the core *N*-(4-phenylthiazol-2-yl)benzamide structure. As such, the scope of the compounds that comprise Claim 38 are enabled because, as described herein, their preparation is within the realm of those of ordinary skill, and their common formula is defined by a particular core structure.

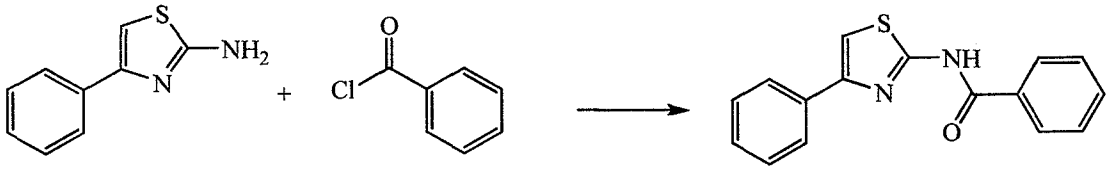
The Office Action also states "claims 40 and 41 encompass variants of IBT13131 and IBT14664...the specification has failed to provide objective evidence that said core structure is the minimal structure require[d] for inhibition of the Hec1-Nek2 interaction." Applicants believe that cancellation of Claims 40 and 41 overcomes this reason for the Office Action's rejection of the claims.

At page 6, the Office Action states "[i]n order to conform to 112, first paragraph, it is necessary for the specification to teach how to make the molecules and ligands of the invention." It is well settled that "[n]ot every last detail [of an invention need] be described [in a patent specification], else patent specifications would turn into production specification, which they were never intended to be." ***In re Gay*, 309 F.2d 769, 774, 135 U.S.P.Q. 311, 316 (C.C.P.A. 1962)**. Indeed, a specification need not describe – and best omits – that which is well known in the art. ***In re Buchner*, 929 F.2d 660, 661, 18 U.S.P.Q.2d 1331, 1332 (Fed. Cir. 1991).**

Applicants attach herewith a copy of a journal article by Dodson R.M. *et al.*, "The Reaction of Ketones with Halogens and Thiourea" *J. Am. Chem. Soc.*, Vol. 67, 2242 (1945). In that paper, the authors describe the preparation of 4-phenyl-2-aminothiazoles according to the following scheme:



As such, the method for forming a 4-phenyl-substituted 2-aminothiazole is well established and by the simplicity of the procedure as described on page 2242, the skill for conducting this reaction is within the realm of an undergraduate student. The next step, acylation of the amino group can be accomplished by the equally routine step according to the following scheme:



In the above reactions, benzophenone and benzoyl chloride can be substituted for by readily available benzophenones and benzoic acid derivatives having substitutions at various position of the benzene rings. As such, the preparation of the disclosed *N*-(4-phenylthiazol-2-yl)benzamide compounds is enabled.

The Office Action has rejected Claims 1-5, 8, 11-20, 36, and 38-41 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Applicants respectfully disagree.

As it relates to Claim 36, the Office Action states that "[Claim 36] encompass[es] molecules or ligands which are not limited to the IBT series of molecules in claim 37." Claim 36 has been canceled. As such, the Office Action's rejection of Claim 36 is moot.

Likewise, Claim 38 now recites a composition that comprises the "compounds that comprise a core *N*-(4-phenylthiazol-2-yl)benzamide structure" that are found to affect a function of Hec1 protein, Nek2 protein and/or Hint1protein involved in cell proliferation as determined by the method of Claim 21. As such, Claim 38 satisfies the written description requirement.

The Office Action states that Claim 40 and 41 "encompass small molecule drugs which are substantially similar to IBT13131 and IBT14664" and that the "description of IBT13131 and IBT 14664 fails to adequately describe the genus of molecules that are substantially similar to IBT13131 and IBT14664." Claims 40 and 41 have been canceled. As such, the Office Action's rejection of Claim 40 and claim 41 is moot.

Reconsideration and withdrawal of the rejections of the claims under35 U.S.C. § 112, first paragraph, is therefore respectfully requested.

REJECTION UNDER 35 U.S.C. § 102(b)

The Office Action has rejected Claims 21, 22, 26, 28, 29, 32, 33, 35, and 36 under 35 U.S.C. § 102(b), as allegedly being anticipated by WO98/45433 (hereinafter "Clark"). Applicants believe the amendments to Claim 21 overcome this rejection.

The Office Action did not reject Claim 23. As such, Applicants have amended Claim 21 to incorporate the limitation of Claim 23, now canceled, that the method identifies a compound that "reduces an interaction between Hec1 protein and Hint1 protein." Therefore, Applicants believe that Claim 21 and those claims that depend therefrom are novel over Clark.

The Office Action has rejected Claims 28, 30 and 31 under 35 U.S.C. § 102(b), as allegedly being anticipated by U.S. 5,516,775 (hereinafter "Zimmermann"). Applicants believe the amendment to Claim 28 overcomes this rejection.

In the present rejection, the Office Action states "[i]t is noted that the recitation of a "method for identifying a molecule that interferes with a function of Hec1 protein, Nek2 protein

and/or Hint1 protein and inhibits cell proliferation" has not been given patentable weight because the recitation occurs in the preamble." Applicants have amended Claim 28 to include the recitation of the preamble in step (b). Therefore, Claims 28, 30 and 31 are novel over Zimmermann because Zimmermann does not disclose a method which specifically tests for a molecule that can interfere with a function of Hec1 protein, Nek2 protein and/or Hint1 protein as it relates to inhibiting cell proliferation.

Reconsideration and withdrawal of the rejection of the claims under35 U.S.C. § 102(b), is therefore respectfully requested.

REJECTION UNDER 35 U.S.C. § 103(a)

The Office Action has rejected Claims 21, 22, 24, 25, 28, 29, and 32-35 under 35 U.S.C. § 103(a), as allegedly being obvious over Clark in view of WO03/050281 (hereinafter "Chumakov"). Applicants respectfully disagree.

The Office Action states that Clark teaches "that an understanding of the molecular events of mitosis will lead to the identification and development of agents to control cell proliferation (page 3, lines 28-30)." This statement is taken from the last paragraph of the Background of the Invention and serves merely as a segue from the Background to the Summary of the Invention. It goes without saying that more knowledge could lead to agents that control cell proliferation. As such, this recitation only restates the obvious that understanding how an aberrant cell works would lead to preventing the aberration.

The Office Action also states that Clark teaches that "HEC may function as an adaptor molecule through the long leucine heptad repeats (page 4, lines 23-24)." What Clark does disclose at page 44, lines 18-21, is:

> The effects on mitosis of overexpression of the HEC mutant indicated that the leucine hepatad repeats of HEC were critical to the protein's function. To explore the potential biochemical basis for the abnormal mitosis after HEC inactivation, the inventors searched for proteins with which HEC interacts.

Table 3 which follows on page 46, shows binding of Nek2 as a function of β-galactosidase activity relative to the control host yeast Y153 alone. These data merely show that the leucine hepatad repeats of HEC are critical to the protein's function. This disclosure does not

contemplate testing for a small molecule's ability to disrupt the interaction between HEC and Hint1.

The Office Action further states "Chumakov et al teach that the screening by ELISA for one or more antagonists which block the binding between two polypeptides is well known in the art." As such, the Office Action uses the Chumakov reference to merely establish the fact that the ELISA test is known and can be used in the disclosed methods.

As it relates to Claim 21, Clark does not teach or suggest the interaction of Hec1 protein and Hint1 protein. As such, the disclosure of Chumakov is irrelevant because the suggestion of ELISA for testing purposes would not create the suggestion to measure the interaction between Hec1 protein and Hint1 protein.

As it relates to the rejection of Claim 28, the Office Action has failed to specifically point out how the combination of Clark and Chumakov renders Claim 28 obvious. Applicants cannot rebut a rejection that is not specifically explained.

The Office Action has rejected Claims 21, 22, 27-29, and 32-35 under 35 U.S.C. § 103(a), as allegedly being obvious over Clark in view of U.S. 6,613,531 (hereinafter "Burgessi"). Applicants respectfully disagree.

The Office Action states that "Burgess et al teach a method wherein an agent which inhibits the binding of a subunit to a polymerase can be identified by determining the proximity of a first label for the subunit and the second label for the polymerase, the first and second labels being different (claims 3 and 4)." Again, the Office Action provides a reference which merely establishes the existence of a known method.

As it relates to Claim 21, Clark does not teach or suggest the interaction of Hec1 protein and Hint1 protein. As such, the disclosure of Burgess is irrelevant because the suggestion that a first and second label to determine the proximity of two different subunits of a polymerase, would not create the suggestion to measure the interaction between Hec1 protein and Hint1 protein.

As it relates to the rejection of Claim 28, the Office Action has failed to specifically point out how the combination of Clark and Burgess renders Claim 28 obvious. Applicants cannot rebut a rejection that is not specifically explained. Moreover, there are no claims dependent from

Claim 28 which read on the use of the co-localization of labels specific for Hec1 and the recited proteins, which is the subject matter that the Office Action found relevant in Burgess.

Reconsideration and withdrawal of the rejection of the claims under35 U.S.C. § 103(a), is therefore respectfully requested.

THE CLAIMS ARE NOW IN CONDITION FOR ALLOWANCE

Claims 1, 11-16, 21, 22, 24-31, 37-39 and 42 are pending in the application. By the amendments made herein and in light of the appended Remarks, Applicants believe the Claims are now in condition for allowance. The Office is encouraged to contact the undersigned Agent to discuss any further issues remaining.

CONCLUSION

Enclosed herewith is payment in the amount of $555.00 under 37 C.F.R. 1.17(a)(3) for a Three-Month Extension of time (Small Entity). This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

BALLARD SPAHR ANDREWS & INGEROLL, LLP

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CERTIFICATE OF ELECTRONIC TRANSMISSION UNDER 37 C.F.R. § 1.8			
I hereby certify that this correspondence, including any items indicated as attached or included, is being transmitted via electronic transmission via EFS-Web on the date indicated below.			
Name of Person Signing (Print/Type)	Richard S. Echler		
Signature	/ Richard S. Echler /	Date	August 21, 2009

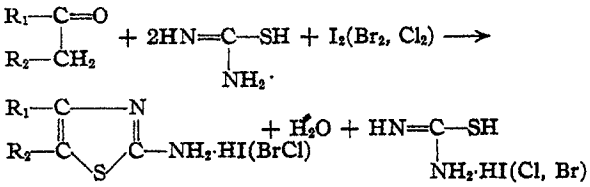
[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF NORTHWESTERN UNIVERSITY]

The Reaction of Ketones with Halogens and Thiourea[1]

BY R. M. DODSON AND L. CARROLL KING

In a study of the reactions of compounds containing reactive hydrogen atoms with halogens in the presence of a base, it was observed that ketones react with thiourea and a halogen[2] to give substituted 2-aminothiazoles.

The reaction may be formulated as



R_1 = methyl-, phenyl-, *m*-nitrophenyl-
R_2 = H-, methyl-, carbethoxy-

In order to establish the generality of the above reaction it was used to prepare thiazoles from a variety of ketones, namely, acetophenone, propiophenone, *m*-nitroacetophenone, acetone and ethyl acetoacetate. In each case the thiazole obtained from the reaction mixture agreed in chemical and physical properties with the data reported in the literature for the corresponding substance obtained by other methods. The structure of the substance described as 2-amino-4-phenyl-5-methylthiazole depends on its formation according to the above reaction and on correct elementary analysis of the compound and its acetyl derivative.

A synthesis of each of the substituted 2-aminothiazoles under consideration was carried out using each of the common halogens. The yield of thiazole varied significantly with the halogen used. The yields, properties and analyses of the thiazoles prepared in this study are listed in Table I.

The general procedure for effecting the reaction may be illustrated by several examples.

To a slurry consisting of 0.2 mole of acetophenone and 0.4 mole of thiourea, 0.2 mole of halogen was added; when iodine was used it was added all at once, bromine was added dropwise, chlorine in weighed amount was distilled into the reaction mixture. After this addition, the reaction mixture was heated overnight on the steam-bath. It was then diluted with water, heated until most of the solid had gone into solution, filtered,[3] cooled and made alkaline with concentrated ammonium hydroxide. The precipitated 2-amino-4-phenylthiazole was separated and crystallized from ethyl alcohol to constant melting point.

For the preparation of 2-amino-4-methylthiazole from acetone, 0.5 mole of halogen was added to a suspension of 1 mole of thiourea in 100 ml. of acetone. After the addition was complete, the reaction mixture was refluxed for two hours; the reflux condenser was then removed and the open vessel heated on the steam-bath overnight. The 2-amino-4-methylthiazole was recovered from the reaction mixture according to the directions of Byers and Dickey.[4]

The acetyl derivatives of these thiazoles were prepared by the reaction of acetic anhydride on about 1 g. of each of the substituted 2-aminothiazoles, the products being recrystallized from ethyl alcohol.

The above reaction can be carried out in a much shorter time if the reaction mixture is heated strongly until a vigorous reaction begins. However, this procedure results in a lowered yield of the thiazole and is accompanied by the separation of much free sulfur and by olfactory evidence of mercaptans. In order to obtain the yield of

TABLE I

Thiazole	Formula	M. p., °C./ Found	M. p., °C. Reported	N analyses, % Found	Calcd.	% Yield[g] obtained using Cl_2	Br_2	I_2
2-Amino-4-methyl-	.....	.....	42[a]	...	...	51	36	77
Acetyl derivative	$C_6H_8N_2OS$	136–137	134[a]	18.48	17.94			
Picrate		229–230 (dec.)	229.5[b]					
2-Amino-4-methyl-5-carbethoxy-	$C_7H_{10}N_2O_2S$	176–177	175[c]	14.89	15.05	60	82	63
Acetyl derivative	$C_9H_{12}N_2O_3S$	225–225.5	[e]	12.31	12.28			
2-Amino-4-phenyl-	$C_9H_8N_2S$	151–152	147[a]	15.84	15.90	49	85	94
Acetyl derivative	$C_{11}H_{10}N_2OS$	214–214.5	208[a]	12.74	12.84			
2-Amino-4-(3-nitrophenyl)-	$C_9H_7N_3O_2S$	188–190	188–191[d]	19.57	19.00	75	95	52
Acetyl derivative[h]	$C_{11}H_9N_3O_3S$	308–313 (dec.)	310–314[d]	17.75 17.65[h]	15.96			
-Amino-4-phenyl-5-methyl-	$C_{10}H_{10}N_2S$	116–118	[e]	14.36	14.73	68	84	94
Acetyl derivative	$C_{12}H_{12}N_2OS$	223	[e]	12.23	12.07			

[a] V. Traumann, *Ann.*, **249**, 31 (1888). [b] E. Otiai, *C. A.*, **33**, 3791[4] (1939). [c] M. Steude, *Ann.*, **261**, 22 (1891). [d] N. Kharasch, Ph. D. Thesis, Northwestern University, 1944, p. 65. [e] Not previously reported. [f] All melting points were observed on a Fischer–Jones melting point block. [g] Based on the ketone, or on the halogen added to the reaction mixture except in the case of 2-amino-4-methylthiazole which is based only on the halogen. [h] This substance has a surprisingly low solubility in most solvents.

(1) For other papers in this series, see L. C. King, THIS JOURNAL, **66**, 894 (1944); 1612 (1944).

(2) The use of bromine to form benzothiazoles from substituted phenylthioureas has been reported by A. Hugershoff, *Ber.*, **36**, 3121 (1903); L. M. White and R. Q. Brewster, *C. A.*, **34**, 5447 (1940); H. Erlenmeyer and H. Ueberwasser, *Helv. Chim. Acta*, **23**, 328 (1940); M. Dyson and T. Harrington, *J. Chem. Soc.*, 374 (1942).

(3) A small amount of free sulfur separated.

(4) J. R. Byers and J. B. Dickey, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 31.

thiazole indicated, 2 moles of thiourea must be present in the reaction mixture for each mole of halogen. In an experiment where 1 mole of thiourea was used for each mole of halogen the yield of thiazole was poor and the product was difficult to purify.

Preliminary experiments indicate that the above reaction is a convenient general synthetic method for preparation of substituted thiazoles. Extension of this reaction to other ketones and to thioamides is in progress.

Summary

It has been demonstrated that acetophenone, propiophenone, *m*-nitroacetophenone, acetone and ethyl acetoacetate react directly with 1.0 mole of a halogen and 2.0 moles of thiourea to give in excellent yield, 2-amino-4-phenylthiazole, 2-amino-4-phenyl-5-methylthiazole, 2-amino-4-(3-nitrophenyl)-thiazole, 2-amino-4-methylthiazole, and 2-amino-4-methyl-5-carbethoxythiazole, respectively.

EVANSTON, ILLINOIS RECEIVED SEPTEMBER 28, 1945

[CONTRIBUTION FROM THE BIOCHEMICAL INSTITUTE AND THE CLAYTON RESEARCH FOUNDATION, THE UNIVERSITY OF TEXAS]

Derivatives of Sulfanilamide. I. N^4-(*p*-Aminobenzoyl)-sulfanilamide and Related Compounds

BY EDITH JU-HWA CHU[1]

Since the discovery and establishment of antagonism between sulfonamide drugs and *p*-aminobenzoic acid, a normal constituent of cells, a number of *p*-aminobenzoic acid derivatives and analogs have been investigated and described. Hirsch[2] demonstrated that *p*-aminobenzamide also possessed bacteriostatic properties, while Johnson and co-workers[3] indicated that in the molecule of *p*-aminobenzoic acid variation of the carbonyl group by replacement or by derivative formation might give compounds exhibiting *p*-aminobenzoic acid activity, bacteriostatic activity, or neither. It was interesting to study the physiological action of a combination of sulfanilamide and *p*-aminobenzoic acid in a simple molecule. The present paper reports syntheses of N^4-(*p*-aminobenzoyl)-sulfanilamide and related compounds and their action on several organisms.

N^4-(*p*-Aminobenzoyl)-sulfanilamide and analogs from albucid, sulfapyridine, sulfadiazine, sulfathiazole and sulfaguanidine have been synthesized by reduction of corresponding nitro derivatives. The most suitable reducing agent is Raney nickel in alcohol or pyridine. N^4-(*p*-Nitrobenzoyl)-sulfanilamide,[4] N^4-(*p*-nitrobenzoyl)-albucid[5] and N^4-(*p*-nitrobenzoyl)-sulfapyridine[6] were previously reported.

These compounds have been tested on *Lactobacillus arabinosus 17-5*, *Streptococcus lactis R*, *Staphylococcus aureus* and *Escherichia coli* and found to be more or less toxic to these organisms; but the action is not reversed by presence of *p*-aminobenzoic acid in most cases.

(1) On leave from the University of Peking, China. The present address is the Department of Medicine, University of Minnesota, Minneapolis, Minn.

(2) J. Hirsch, *Science*, **96**, 140 (1942).

(3) O. H. Johnson, D. E. Green and R. Pauli, *J. Biol. Chem.*, **153**, 37 (1944).

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Preparation and Properties

N^4-(*p*-Nitrobenzoyl)-sulfanilamide and Analogs.—A mixture of one millimole each of *p*-nitrobenzoyl chloride and sulfanilamide in 5 ml. of dry pyridine was refluxed for an hour, cooled and then poured into ice water. The precipitate thus obtained was recrystallized from acetic acid or pyridine, yielding pale yellow fine needles. It is difficultly soluble in benzene or 1,4-dioxane, slightly soluble in acetic acid, acetone or alcohol, moderately soluble in isobutyl acetate, and soluble in pyridine, ethanolamine, diethanolamine and triethanolamine. It is recovered unchanged by boiling with 10% sodium hydroxide or concentrated hydrochloric acid for ten–fifteen minutes, but is hydrolyzed by refluxing with 10% sodium hydroxide for two hours, *p*-nitrobenzoic acid being identified. It was also synthesized from *p*-nitrobenzanilide by treatment with chlorosulfonic acid and reaction of the aromatic sulfonyl chloride with ammonium hydroxide; yield, 75%.

Analogs were prepared from albucid, sulfapyridine, sulfathiazole, sulfadiazine and sulfaguanidine, respectively. N^4-(*p*-Nitrobenzoyl)-albucid was also prepared by acetylation of N^4-(*p*-nitrobenzoyl)-sulfanilamide with acetic anhydride and pyridine in a quantitative yield.

N^4-(*p*-Aminobenzoyl)-sulfanilamide and Analogs.—The most satisfactory means for reducing N^4-(*p*-nitrobenzoyl)-sulfanilamide thus far tried is Raney nickel in alcohol or pyridine. A mixture of 2 g. of N^4-(*p*-nitrobenzoyl)-sulfanilamide and 10 g. of Raney nickel in 20 ml. of alcohol was refluxed on a steam-bath for an hour and then filtered. The precipitate of N^4-(*p*-aminobenzoyl)-sulfanilamide was recrystallized from acetone. It melts at 276° first, solidifies and then melts again at 313° dec.

N^4-(*p*-Nitrobenzoyl) derivatives of sulfathiazole, sulfapyridine, sulfadiazine and sulfaguanidine were similarly reduced to amino derivatives by Raney nickel except that pyridine was used as the solvent instead of alcohol and the product was washed with acetic acid.

N^4-(*p*-Aminobenzoyl)-sulfathiazole was insoluble in most solvents and difficultly purified and the analysis of nitrogen content always was 2% lower. It was acetylated to acetyl derivative, prisms, m. p. 314° dec.

Physiological Action on Microörganisms.—These compounds have been tested on *Lactobacillus arabinosus 17-5*, *Streptococcus lactis R*, *Staphylococcus aureus* and *Escherichia coli*, respectively. For testing with *Lactobacillus arabinosus 17-5* a medium described by Lewis[7] was modified by

(7) J. C. Lewis, *J. Biol. Chem.*, **146**, 441 (1942).